

### **Listing of Claims:**

The listing of claims will replace all prior versions and listings of claims in the application:

Claim 1 (currently amended) A method for designing primer pairs for amplifying a target nucleic acid sequence using a reference sequence, comprising the steps of:

choosing ~~a~~said reference sequence;

removing at least selected repeat regions in said reference sequence ~~to~~thereby yielding removed and unremoved regions of said reference sequence;

selecting primer sequences from said unremoved regions of said reference sequence according to two or more parameters including primer length and primer melting temperature to yield a set of primers;

evaluating said set of primers for extent of coverage and overlap of said reference sequence; and

selecting a subset of primer pairs having reduced overlap from said set of primers, ~~wherein if said primers were used to amplify said reference sequence such that amplicons corresponding to said subset of primer pairs, the resulting amplified portions of said reference sequence would overlap by less than about 5%, and further, wherein said subset of primer pairs in said subset may be used have been thereby designed~~ for amplifying said target nucleic acid sequence.

Claim 2 (original) The method of claim 1, wherein said primer length is selected to be between about 28 nucleotides and about 36 nucleotides.

Claim 3 (original) The method of claim 1, wherein said primer melting temperature is between about 72 °C and about 88 °C.

Claim 4 (original) The method of claim 1, wherein said two or more parameters from said first selecting step is selected from the group of stringency, duplex existence, specificity, GC clamp, hairpin existence, sequence repeat existence, dissociation minimum for 3' dimer, dissociation minimum 3' terminal stability range, dissociation minimum for minimum acceptable loop, percent maximum homology, percent consensus homology, maximum number of acceptable sequence repeats, frequency threshold, and maximum length of acceptable dimers.

Claim 5 (original) The method of claim 1, wherein said extent of coverage is above about 90% of said reference sequence.

Claim 6 (cancelled)

Claim 7 (original) The method of claim 1, wherein said removing step is performed by a computer program.

Claim 8 (cancelled)

Claim 9 (original) The method of claim 1, wherein said first selecting step is performed by a computer system.

Claim 10 (cancelled)

Claim 11 (currently amended) The method of claim 1, wherein said second selecting step selects a subset of primer pairs with a ~~minimal or substantially minimal number of primer pairs required to amplify said target sequence~~ lower cost than any other subset of primer pairs from said set of primers.

Claim 12 (original) The method of claim-11 1, wherein said second selecting step selects a subset of primer pairs with a least number of primer pairs required to amplify said target sequence.

Claim 13 (original) The method of claim-11 1, wherein said second selecting step selects said subset of primer pairs according to at least one parameter selected from the group of overlap length, gaps between pairs of primer pairs, and necessity of adding another primer pair to the subset.

Claim 14 (original) The method of claim 1, 11, 12 or 13, wherein said second selecting step is performed by a computer system.

Claim 15 (currently amended) The method of claim-11 1, wherein said a computer program executes a single-source shortest-path algorithm to select said subset of primer pairs.

Claim 16 (original) The method of claim 15, wherein said computer program executes an algorithm solving a single-source shortest path problem on a weighted, directed graph  $G=(V,E)$  for the case in which all edge weights are nonnegative, and  $w(u,w) \geq 0$  for each edge  $(u,v) \in E$ .

Claim 17 (original) The method of claim-11 1, wherein said a computer program executes a greedy algorithm to select said subset of primer pairs.

Claim 18 (original) The method of claim 1, wherein said target sequence is genomic DNA from a human species.

Claim 19 (original) The method of claim 1, wherein said target sequence is genomic DNA from a non-human primate species.

Claim 20 (original) The method of claim 1, wherein said reference sequence is genomic DNA from a human species.

Claim 21 (original) The method of claim 1, wherein said primer length is about 28 nucleotides to about 36 nucleotides and said melting temperature is about 72 °C to about 88 °C.

Claim 22 (currently amended) A computer-implemented method for designing primer pairs for amplifying a target nucleic acid sequence comprising:

inputting a reference sequence into a computer program, wherein said computer program is designed to recognize regions that are repeated in said reference sequence;  
removing selected repeat regions in said reference sequence to yield removed and unremoved reference sequence;  
selecting primer sequences from said unremoved reference sequence according to two or more parameters including primer length and primer melting temperature to yield a set of primers;  
evaluating said set of primers for extent of coverage and overlap of said reference sequence; and  
selecting a subset of primer pairs having reduced overlap from said set of primers such that amplicons corresponding to said subset of primer pairs overlap by less than about 5%, and wherein said ~~subset of primer pairs in said subset may be used~~ have been thereby designed for amplifying said target nucleic acid sequence.

Claim 23 (previously presented) The method of claim 22, wherein said removing step is performed by said computer program, and wherein said computer program further references a database.

Claim 24 (cancelled)

Claim 25 (cancelled)

Claim 26 (previously presented) The method of claim 22, wherein said selecting primer sequences in said first selecting step uses additional parameters selected from the group of stringency, duplex existence, specificity, GC clamp, hairpin existence, sequence repeat existence, dissociation minimum for 3' dimer, dissociation minimum 3' terminal stability range, dissociation minimum for minimum acceptable loop, percent maximum homology, percent consensus homology,

maximum number of acceptable sequence repeats, frequency threshold, and maximum length of acceptable dimers.

Claim 27 (cancelled)

Claim 28 (currently amended) The method of claim 22, wherein a second computer program executes an algorithm that in said second selecting step selects a subset of primer pairs according to at least one parameter selected from the group of overlap length, gaps between pairs of primer pairs, and necessity of adding another primer pair to the subset ~~with a minimal or substantially minimal number of primer pairs required to amplify said target sequence.~~

Claim 29 (previously presented) The method of claim 22, wherein in said second selecting step a second computer program executes an algorithm that selects said subset of primer pairs according to at least one parameter selected from the group of overlap length, gaps between pairs of primer pairs, and necessity of adding another primer pair to the subset.

Claim 30 (previously presented) The method of claim 22, wherein in said second selecting step a second computer program executes a single-source shortest-path algorithm.

Claim 31 (previously presented) The method of claim 22, wherein in said second selecting step a second computer program executes Dijkstra's algorithm.

Claim 32 (currently amended) A system that designs primer pairs for amplifying a target nucleic acid sequence comprising:

a processor; and

a computer readable medium coupled to said processor for storing a computer program comprising: computer code that receives input of a reference sequence; computer code that removes at least selected repeat regions in said reference sequence to yield removed and unremoved reference sequence; computer code that selects primer sequences from said unremoved reference sequence according to two or more parameters including primer length and primer melting temperature to yield a set of primers; computer code that evaluates said set of primers for extent of coverage and overlap of said reference sequence; and computer code that selects a subset of primer pairs having reduced overlap from said set of primers such that amplicons corresponding to said subset of primer pairs overlap by less than about 5%, and wherein said primer pairs in said subset have been thereby designed for amplifying said target nucleic acid sequence.